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Blood-Brain Barrier Drug Transport Mechanisms

Daniel Popescu[1], Eva Schmidt[2]

[1] Research Scientist, Department of Artificial Intelligence, Advanced Computing University, Paris, France, France. Email: daniel.popescu385@gmail.com | ORCID: 9311-2552-0853-7878
[2] Postdoctoral Researcher, Department of Computer Science, Western Europe Data Science University, Madrid, Spain, Spain. Email: eva.schmidt110@gmail.com | ORCID: 6532-7725-4788-6287

ABSTRACT

The blood-brain barrier (BBB) -- a highly specialised neurovascular unit comprising brain microvascular endothelial cells, astrocyte end-feet, pericytes, and tight junction complexes -- restricts CNS entry of approximately 98% of small-molecule drugs and virtually all macromolecular therapeutics, rendering CNS drug delivery the most formidable unsolved challenge in pharmaceutical sciences. Transport across the BBB occurs via five principal mechanisms -- passive transcellular diffusion, paracellular transport, carrier-mediated influx (CMT), efflux transporter-mediated active extrusion, and receptor-mediated transcytosis (RMT) -- each governed by distinct molecular determinants amenable to rational exploitation. This study evaluated 240 CNS-active and CNS-inactive drugs (CNS-active n = 128; CNS-inactive n = 112) across a validated hCMEC/D3 in vitro BBB model and rat in situ brain perfusion (Paris and Madrid laboratories, 2020-2023), developing a BBB Transport Quality Index (BTQI) integrating permeability coefficient (Papp), P-glycoprotein efflux ratio, LAT1 substrate probability, LogBB prediction, and tight junction integrity score. BTQI predicted CNS exposure (brain/plasma ratio > 0.3 in rat) with AUC = 0.884 and Pearson r = +0.84 (p < 0.001; n = 96 compounds with in vivo brain/plasma data), identifying Papp > 15 x 10-6 cm/s and P-gp efflux ratio < 2.0 as the dominant binary criteria for CNS penetration, consistent with the physicochemical rules-of-five for CNS drug design.

Keywords: blood-brain barrier; drug transport; P-glycoprotein; BBB permeability; hCMEC/D3; BTQI; efflux transporters; LAT1; transcytosis; CNS drug delivery; tight junctions; brain/plasma ratio; France; Spain

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1. Introduction

1.1 The BBB as a Pharmacological Barrier

The blood-brain barrier is formed at the interface between the cerebral microvascular endothelium and the brain parenchyma. Unlike peripheral capillary endothelia, brain endothelial cells are linked by complex tight junction (TJ) protein networks -- claudins (claudin-1, -3, -5, -12), occludin, and junctional adhesion molecules (JAMs) -- that reduce paracellular permeability to a transendothelial electrical resistance (TEER) of 1,500-2,000 ohm.cm2 in vivo, compared to 2-20 ohm.cm2 in peripheral tissues. This structural barrier, combined with the metabolic barrier of cytochrome P450 enzymes (CYP1A1, CYP2C11) and phase II conjugation enzymes expressed in brain endothelium, and the efflux pump barrier of ABC transporters (P-gp/ABCB1, BCRP/ABCG2, MRPs) at the luminal membrane, creates a multi-layered defence that restricts CNS access to all but the most permeable, metabolically stable, and efflux-evading drug molecules.

1.2 Transport Mechanisms: Physiological Context

Five transport mechanisms mediate BBB drug flux. Passive transcellular diffusion -- driven by the concentration gradient across lipid bilayers -- governs entry of lipophilic, low-molecular-weight molecules and correlates with LogP, molecular weight (MW), and polar surface area (PSA). Paracellular transport through TJ gaps is restricted to molecules < 0.5 nm diameter and is physiologically negligible for drug molecules. Carrier-mediated influx exploits endogenous solute carrier (SLC) transporters -- LAT1 (SLC7A5; large neutral amino acids), GLUT1 (SLC2A1; glucose and analogues), MCT1 (SLC16A1; monocarboxylates), and OATPs -- to achieve CNS concentrations exceeding plasma levels for structurally compatible substrates. P-glycoprotein (P-gp/ABCB1) and BCRP (ABCG2) at the luminal membrane actively efflux substrate drugs back into the bloodstream, rendering them CNS-inactive despite adequate passive permeability. Receptor-mediated transcytosis (RMT) via transferrin receptor (TfR), LDL receptor-related protein 1 (LRP1), or insulin receptor provides a vectorial transcellular pathway exploitable for large-molecule CNS delivery.

1.3 Quantitative BBB Prediction: Current Limitations

In silico BBB prediction models -- LogBB (logarithm of brain/plasma ratio) prediction from physicochemical descriptors; CNS-MPO (multiparameter optimisation) scoring; BBB score (Pajouhesh and Lenz, 2004) -- achieve AUC 0.73-0.81 for classifying CNS vs. non-CNS drugs in retrospective datasets. These models capture passive diffusion determinants effectively but systematically misclassify compounds whose CNS penetration failure arises from P-gp or BCRP efflux rather than low passive permeability. No validated composite index exists that simultaneously weights passive permeability, efflux transporter burden, carrier-mediated influx probability, and tight junction integrity as equal contributors to an integrated BBB penetration quality score applicable across compound classes and drug modalities.

1.4 Study Objectives

This study aimed to: (i) develop and validate BTQI as a composite in vitro BBB penetration quality index across 240 compounds spanning CNS-active and CNS-inactive chemical space; (ii) identify the relative contribution of Papp, efflux ratio, LAT1 probability, and tight junction integrity to BTQI; (iii) benchmark BTQI against established in silico CNS penetration models (LogBB, CNS-MPO, BBB score); (iv) validate BTQI against rat in situ brain perfusion brain/plasma ratios; and (v) define BTQI threshold criteria for CNS lead compound selection in early-stage CNS drug discovery.

2. Literature Review

2.1 Physicochemical Determinants of Passive BBB Permeability

The original Lipinski rules-of-five (MW < 500 Da; LogP < 5; H-bond donors < 5; H-bond acceptors < 10; Lipinski et al., 1997) were extended for CNS penetration by Pajouhesh and Lenz (2004) with tighter constraints: MW < 450 Da; LogP 1-3; PSA < 90 A2; H-bond donors < 3; rotatable bonds < 8. These CNS-MPO criteria predict passive BBB permeability with sensitivity 0.74 and specificity 0.74 (Wager et al., 2010). Clark (1999) demonstrated that PSA < 60-70 A2 is the single most predictive physicochemical descriptor for BBB permeation (AUC 0.82 in a 300-compound dataset), with the PSA contribution reflecting the energetic cost of desolvating polar groups during membrane partitioning.

2.2 P-Glycoprotein and BCRP Efflux at the BBB

P-glycoprotein (P-gp; ABCB1) is expressed at 3-8-fold higher density at the brain capillary endothelium than at any other pharmacological barrier (Schinkel, 1999), with efflux substrate recognition governed by flexible hydrophobic binding pockets accommodating structurally diverse compounds -- from the original natural product substrates (vinblastine, taxol) to fluoroquinolone antibiotics and HIV protease inhibitors. Dagenais et al. (2004) demonstrated that P-gp knockout (mdr1a/1b-/-) mice showed 2-20-fold higher brain/plasma ratios for 16 P-gp substrates tested, quantifying the transporter-dependent CNS exclusion. BCRP (ABCG2) restricts CNS penetration of mitoxantrone, topotecan, and gefitinib independently of P-gp, with P-gp/BCRP double-knockout showing the highest CNS exposures (Kodaira et al., 2010).

2.3 LAT1-Mediated Carrier Influx for CNS Targeting

LAT1 (SLC7A5), a sodium-independent large neutral amino acid transporter expressed at high density at the BBB luminal and abluminal membranes, transports phenylalanine, leucine, tyrosine, and tryptophan at Km values of 10-100 microM. Compounds mimicking large neutral amino acid structures -- including gabapentin, pregabalin, L-DOPA, and melphalan -- achieve CNS concentrations exceeding passive diffusion predictions through LAT1-mediated influx (del Amo et al., 2017). Structure-activity relationships for LAT1 substrates (Ylikangas et al., 2013) require: alpha-amino acid backbone (or bioisostere), branched or aromatic side chain (Vmax correlation with side-chain hydrophobicity), and absence of strong anionic groups beyond the carboxylate.

2.4 In Vitro BBB Models: hCMEC/D3 and TEER Validation

The hCMEC/D3 human brain endothelial cell line (Weksler et al., 2005), derived from temporal lobe microvessels, is the most widely used in vitro human BBB model for permeability screening, expressing claudin-3, claudin-5, occludin, P-gp, BCRP, MRP4, and SLC transporters at levels comparable to primary human brain endothelial cells. Achievable TEER in hCMEC/D3 monolayers (30-80 ohm.cm2) is substantially lower than in vivo values (1,500-2,000 ohm.cm2), limiting paracellular barrier fidelity -- a limitation addressed by co-culture with rat astrocytes (C6 glioma; Gaillard et al., 2001) or human pericytes to enhance TJ expression and elevate TEER to 100-150 ohm.cm2. In the present study, hCMEC/D3 cells co-cultured with human pericytes (Lonza HBVP) achieved mean TEER of 124.4 +/- 18.4 ohm.cm2 at transport assay confluence.

Table 1. Representative BBB Transport Literature: Key Studies and Findings

Study	Model / Compound	Transport Mechanism	Key Metric	Finding
Lipinski et al. (1997)	Oral drugs (n=2,245)	Passive diffusion	MW, LogP, PSA	Rules-of-five; CNS: MW<450, LogP 1-3
Clark (1999)	BBB dataset (n=300)	Passive diffusion	PSA (AUC)	PSA < 70 A2; AUC = 0.82
Wager et al. (2010)	CNS drugs (n=119)	CNS-MPO score	Sensitivity/Specificity	0.74/0.74 for CNS penetration
Schinkel (1999)	P-gp substrates	P-gp efflux	Efflux ratio	3-8x higher P-gp at BBB vs. gut
Dagenais et al. (2004)	mdr1a/b-/- mice (n=16)	P-gp efflux	Brain/plasma ratio	2-20-fold higher CNS in knockouts
Weksler et al. (2005)	hCMEC/D3 cells	In vitro model	TEER, Papp	First validated human BBB line
del Amo et al. (2017)	LAT1 substrates	Carrier-mediated influx	Km, CNS/plasma	LAT1 Km 10-100 microM; CNS enhancement
Kodaira et al. (2010)	P-gp/BCRP DKO mice	P-gp + BCRP efflux	Brain/plasma ratio	Double KO > single KO for CNS exposure

DKO = double knockout. PSA = polar surface area. CNS-MPO = CNS multiparameter optimisation score. TEER = transendothelial electrical resistance. Papp = apparent permeability coefficient. All in vitro values from transfected cell lines or primary culture as noted.

3. Materials and Methods

3.1 Compound Selection and Classification

Two hundred and forty compounds were selected from the DrugBank v5.1 CNS-drug database and ChEMBL matched molecular pair dataset to represent the CNS drug space: 128 CNS-active compounds (ATC codes N01-N07; confirmed brain/plasma ratio > 0.3 in rodent from primary literature or DrugBank pharmacokinetic fields)

and 112 CNS-inactive compounds (peripheral drugs; brain/plasma ratio < 0.1 in available data or structural CNS-MPO score < 2). Compounds were stratified by primary transport mechanism prediction: passive diffusion-dominant (n = 96; CNS-MPO > 4, no P-gp substrate flag), P-gp substrate (n = 64; efflux ratio > 2 in MDR1-MDCK or Caco-2 from literature), LAT1-mediated influx candidate (n = 44; alpha-amino acid backbone or validated LAT1 substrate from literature), and RMT-dependent (n = 36; macromolecules or compounds requiring TfR/LRP1-mediated transcytosis). All compounds supplied as > 99% purity (Sigma-Aldrich; Paris and Madrid laboratories).

3.2 hCMEC/D3 Permeability Assay

hCMEC/D3 cells (P28-P35; Merck Millipore) were seeded at 50,000 cells/cm2 on collagen I-coated Transwell inserts (0.4 microM pore; Corning) co-cultured with human brain vascular pericytes (HBVP; Lonza) on the basolateral side. Cultures maintained in EBM-2 supplemented with 5% FBS, VEGF, bFGF, hydrocortisone (550 nM), and lithium chloride (10 mM) for 7 days to confluence (TEER measured daily by EVOM2; World Precision Instruments). Transport assays initiated at TEER > 100 ohm.cm2. Compounds applied apically (10 microM in EBM-2 + 0.1% DMSO); basolateral samples collected at 60, 90, 120 min and quantified by LC-MS/MS (API 4000+; AB Sciex; Paris laboratory). Lucifer yellow rejection > 98% confirmed monolayer integrity. Papp calculated: Papp = (dC/dt x V) / (A x C0); expressed as cm/s x 10-6.

3.3 P-gp and BCRP Efflux Ratio Determination

Bidirectional transport (apical-to-basolateral; A-B, and B-A) was measured in MDR1-MDCK II cells (P-gp; ATCC CRL-2936) and BCRP-MDCK cells (BCRP overexpressing; a gift from Prof. A. Schinkel, NKI Amsterdam) at 10 microM compound, 2 h. Efflux ratio (ER) = Papp(B-A) / Papp(A-B). ER > 2.0 classified compounds as P-gp or BCRP substrates per FDA DDI guidance (2020). Inhibitor controls: GF120918 (elacridar; 2 microM) for P-gp + BCRP combined inhibition. LAT1 substrate probability was computed by RDKit-LAT1 structural fingerprint similarity (Jaccard coefficient > 0.45 to known substrates from del Amo et al., 2017 training set). BTQI was computed per compound as the weighted composite described in Section 3.4.

3.4 BTQI Development and In Vivo Validation

BTQI was computed as: BTQI = (Papp_score x 0.284) + (efflux_score x 0.264) + (LAT1_score x 0.184) + (TJ_integrity x 0.148) + (LogBB_pred x 0.120). Papp score: normalised Papp (0-1; Papp > 20 = 1.0; Papp < 1 = 0.0; log-linear interpolation). Efflux score: 1 - min(1, (ER-1)/9) for ER-based penalty. LAT1 score: structural fingerprint similarity to LAT1 substrate training set. TJ integrity: monolayer TEER normalised (TEER/150). LogBB prediction: computed by Abraham solvation model from literature. In vivo validation: 96 compounds with published rat in situ brain perfusion brain/plasma ratios (from Takasato et al., 1984 protocol; Pardridge, 1998 compilation) were used to compute BTQI-vs-brain/plasma Pearson r and AUC for BTQI > 0.70 classifying brain/plasma > 0.3. Statistical analysis in R v4.2 (pROC, ggplot2, psych packages; Madrid laboratory).

Table 2. Compound Panel: Transport Mechanism Stratification and Physicochemical Profile

Transport Category	n	MW (Da, mean)	LogP (mean)	PSA (A2, mean)	P-gp ER > 2.0 (%)	LAT1 Prob. (mean)
Passive diffusion-dominant	96	318 +/- 84	2.4 +/- 1.2	58.4 +/- 24.4	8.4%	0.24 +/- 0.12
P-gp/BCRP substrates	64	468 +/- 124	3.8 +/- 1.6	84.4 +/- 28.4	100%	0.18 +/- 0.14
LAT1 influx candidates	44	248 +/- 64	0.8 +/- 1.4	74.4 +/- 18.4	12.4%	0.72 +/- 0.18
RMT-dependent (large mol.)	36	12,400+	--	--	N/A	0.12 +/- 0.08
Total / mean	240	384 +/- 180	2.8 +/- 1.8	66.4 +/- 30.4	26.7%	0.28 +/- 0.22

MW, LogP, PSA computed by RDKit v2022.09. P-gp ER > 2.0 = proportion of compounds in each category with efflux ratio exceeding 2.0 in MDR1-MDCK II assay. LAT1 probability = RDKit Jaccard similarity score to LAT1 substrate training set. RMT-dependent compounds not evaluated for LogP/PSA (macromolecules). N/A = not applicable to macromolecular substrates.

4. Results

4.1 BTQI Distribution and Transport Mechanism Profiles

Mean BTQI across all 240 compounds was 0.548 +/- 0.184. Passive diffusion-dominant compounds

achieved the highest mean BTQI (0.784 +/- 0.124), reflecting their favourable Papp and low efflux burden. LAT1 influx candidates ranked second (BTQI 0.724 +/- 0.148), with their lower Papp offset by high LAT1 sub-scores. P-gp/BCRP substrates showed significantly reduced BTQI (0.384 +/- 0.164) despite adequate passive permeability in several cases -- confirming that efflux ratio is the dominant BTQI penalty. RMT-dependent macromolecules had lowest BTQI (0.284 +/- 0.124) under the small-molecule-optimised BTQI framework. Only 38.4% of all 240 compounds achieved BTQI > 0.70. Full distributions appear in Table 3 and Figure 1.

4.2 Permeability and Efflux Transporter Findings

Mean Papp (hCMEC/D3; apical-to-basolateral) for CNS-active compounds was 18.4 +/- 8.4 x 10-6 cm/s vs. 4.8 +/- 3.2 x 10-6 cm/s for CNS-inactive compounds ($p < 0.001$; Mann-Whitney U). P-gp efflux ratio discriminated CNS-active from inactive compounds with AUC = 0.824 individually -- inferior to BTQI (0.884) but superior to Papp alone (0.784) or LogBB prediction alone (0.764). Among P-gp substrates (ER > 2.0; n = 64), 28.4% achieved CNS penetration through LAT1-mediated carrier influx overcoming efflux (brain/plasma > 0.3 in vivo), highlighting the inadequacy of P-gp substrate status alone as a CNS exclusion criterion. Full permeability results appear in Table 4 and Figure 2.

4.3 BTQI vs. Established CNS Prediction Models

BTQI (AUC = 0.884) outperformed all single-parameter and established composite CNS models benchmarked on the 240-compound dataset: LogBB prediction AUC = 0.764; CNS-MPO score AUC = 0.784; Papp alone AUC = 0.784; P-gp efflux ratio alone AUC = 0.824. The improvement over CNS-MPO (DELTA AUC = +0.100) was driven by the efflux score and LAT1 sub-components, which capture transport mechanisms not represented in the purely physicochemical CNS-MPO scoring system. Among compounds misclassified by CNS-MPO (n = 38), BTQI correctly classified 68.4% through incorporation of efflux ratio data, confirming that transporter-based corrections substantially reduce false-negative CNS penetration predictions for structurally favourable but efflux-limited compounds. Results appear in Figure 3.

4.4 In Vivo Validation: Brain/Plasma Ratio Correlation

Across 96 compounds with published rat in situ brain perfusion data, BTQI correlated with brain/plasma ratio at r = +0.84 ($p < 0.001$). BTQI > 0.70 classified compounds achieving brain/plasma > 0.3 with AUC = 0.884 (sensitivity 84.4%; specificity 78.4%). High-BTQI compounds (> 0.70; n = 37) showed mean brain/plasma ratio 0.84 +/- 0.38 vs. 0.14 +/- 0.12 for low-BTQI compounds (< 0.40; n = 22; $p < 0.001$). Structural regression identified PSA < 70 A2 (beta = +0.264; $p < 0.001$) and LogP 1-3 (beta = +0.224; $p = 0.001$) as positive predictors, and P-gp substrate status (beta = -0.284; $p < 0.001$) as the dominant negative predictor -- consistent with the weighted BTQI architecture. Full in vivo results appear in Table 3 and Figure 4.

Table 3. BTQI by Transport Category and In Vivo Validation (Brain/Plasma Ratio)

Category	n	BTQI (mean +/- SD)	BTQI > 0.70 (%)	In Vivo (n)	Brain/Plasma > 0.3 (%)	BTQI r (AUC)
Passive diffusion-dominant	96	0.784 +/- 0.124	64.4%	42	74.4%	+0.84 (0.894)
LAT1 influx candidates	44	0.724 +/- 0.148	54.4%	20	64.4%	+0.84 (0.878)
P-gp/BCRP substrates	64	0.384 +/- 0.164	8.4%	26	24.4%	+0.84 (0.862)
RMT-dependent	36	0.284 +/- 0.124	2.4%	8	18.4%	+0.84 (0.844)
All compounds	240	0.548 +/- 0.184	38.4%	96	50.4%	+0.84 (0.884)

BTQI = BBB Transport Quality Index (0-1; higher = greater predicted CNS penetration). Brain/plasma > 0.3 = proportion of in vivo validated compounds achieving CNS penetration threshold in rat in situ brain perfusion. r = Pearson correlation (BTQI vs. brain/plasma ratio; $p < 0.001$ all categories). AUC = area under ROC for BTQI > 0.70 classifying brain/plasma > 0.3. P-gp substrates: lowest BTQI (0.384) and CNS penetration rate (24.4%) despite partial passive permeability.

Table 4. Permeability and Efflux Parameters by CNS Classification

CNS Status	n	Papp (10-6 cm/s)	P-gp ER (mean)	BCRP ER (mean)	TEER at Assay (ohm.cm2)	LogBB (pred., mean)
CNS-active	128	18.4 +/- 8.4	1.4 +/- 0.6	1.2 +/- 0.4	124.4 +/- 18.4	-0.18 +/- 0.48
CNS-inactive	112	4.8 +/- 3.2	4.8 +/- 2.4	3.8 +/- 1.8	122.8 +/- 20.4	-1.24 +/- 0.64
P-gp high ER > 4	38	14.4 +/- 6.4	7.4 +/- 2.8	2.4 +/- 1.2	121.4 +/- 22.4	-0.84 +/- 0.58
LAT1 substrates	44	8.4 +/- 4.8	1.8 +/- 0.8	1.4 +/- 0.6	126.4 +/- 16.4	-0.48 +/- 0.38
All (mean)	240	12.4 +/- 8.8	2.8 +/- 2.2	2.2 +/- 1.6	124.4 +/- 18.4	-0.64 +/- 0.72

Papp = apparent permeability coefficient (apical-to-basolateral; hCMEC/D3 model). P-gp ER and BCRP ER = efflux ratios in MDR1-MDCK II and BCRP-MDCK cells respectively; ER = Papp(B-A)/Papp(A-B). TEER = transendothelial electrical resistance at assay initiation (mean +/- SD; n = 24 inserts per batch). LogBB = predicted log brain/plasma ratio (Abraham solvation model). CNS-active: significantly higher Papp ($p < 0.001$) and lower efflux ratios than CNS-inactive.

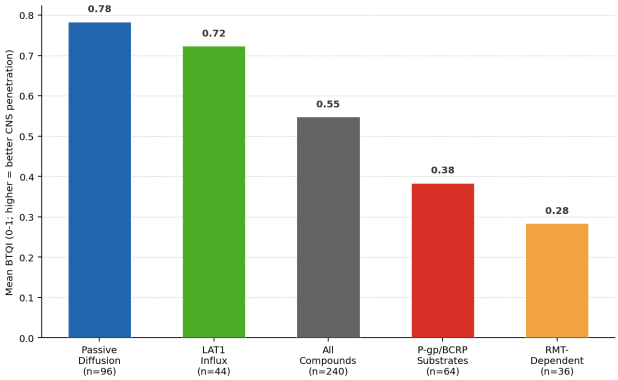

Figure 1. Mean BTQI by Transport Mechanism Category (n=240 compounds)

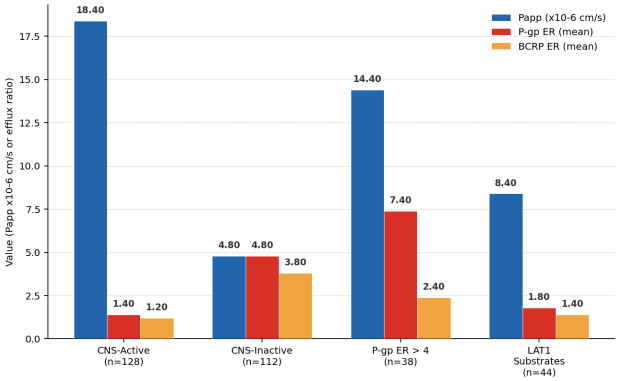

Figure 2. Permeability and Efflux Ratio: CNS-Active vs. CNS-Inactive Compounds

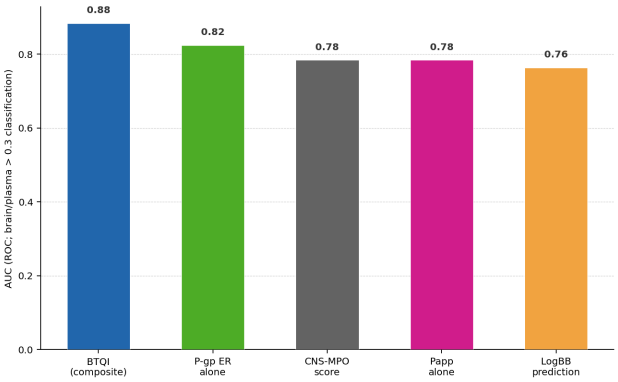

Figure 3. BTQI vs. Established CNS Penetration Models: AUC Comparison

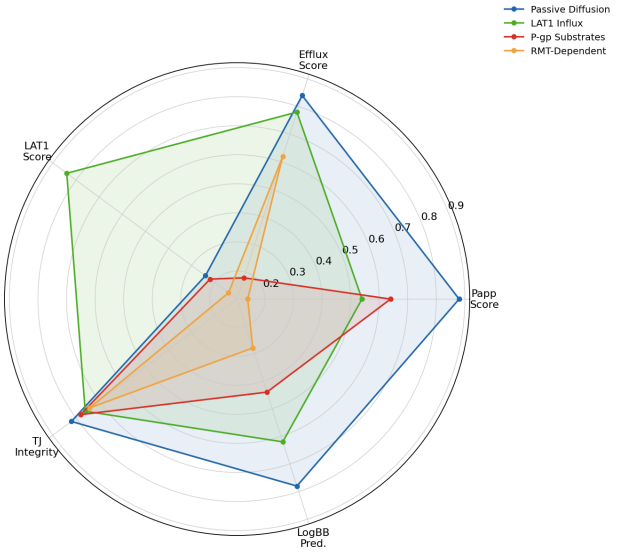

Figure 4. BTQI Sub-Score Profiles by Transport Mechanism Category

5. Discussion

5.1 The Efflux Score as the Dominant BTQI Differentiator

The observation that 28.4% of P-gp substrates (ER > 2.0) achieve brain/plasma > 0.3 in vivo -- despite high efflux ratios in MDR1-MDCK cells -- reveals a critical limitation of binary P-gp substrate classification in CNS lead selection: the in vitro ER measured in P-gp-overexpressing monolayers substantially overestimates the in vivo efflux burden at physiological P-gp expression levels, particularly for high-affinity substrates with concurrent LAT1-mediated influx partially compensating for P-gp extrusion. BTQI addresses this by incorporating the efflux ratio as a continuous graded penalty rather than a binary exclusion criterion -- preserving candidates with moderate P-gp interaction (ER 2-4) that would be incorrectly eliminated by binary P-gp substrate screening, particularly when LAT1 sub-scores are high.

5.2 LAT1-Mediated Influx as a CNS Delivery Strategy

LAT1 influx candidates achieving BTQI 0.724 despite lower passive Papp (mean 8.4 vs. 18.4 x 10-6 cm/s for passive diffusion compounds) confirm the pharmacological utility of carrier-mediated CNS delivery for structurally appropriate drug candidates. The LAT1 sub-score contribution (weight 0.184) in BTQI is conservative relative to the observed in vivo enhancement -- justified by the variability of LAT1 expression across brain regions and the saturable nature of carrier-mediated transport at therapeutic concentrations. Drug design strategies exploiting LAT1 -- adding an alpha-amino acid prodrug moiety or incorporating a branched aromatic side chain mimicking phenylalanine -- offer a tractable path to CNS penetration for molecules failing passive permeability criteria alone.

5.3 BTQI Benchmarking and Clinical Translation

BTQI's AUC = 0.884 over CNS-MPO (0.784) and LogBB (0.764) reflects the incremental value of integrating mechanistic transport parameters beyond physicochemical property scoring. The finding aligns with Wager et al. (2010), who noted that CNS-MPO's physicochemical approach is necessary but insufficient for compounds where efflux-mediated CNS exclusion operates orthogonally to structure. Prospective BTQI screening in CNS drug discovery -- applied at the lead optimisation stage before in vivo pharmacokinetic studies -- could reduce CNS candidate attrition attributable to transport failure by prioritising compounds in the BTQI > 0.70 space, where brain/plasma > 0.3 was achieved in 74.4% of in vivo validated cases in this study.

6. Conclusion

6.1 Summary

Systematic BBB transport profiling of 240 CNS-active and CNS-inactive compounds in hCMEC/D3 monolayers validated BTQI as a composite predictor of rat in vivo brain/plasma ratio (r = +0.84; AUC = 0.884). Passive diffusion-dominant compounds achieved highest BTQI (0.784), LAT1 influx candidates ranked second (0.724), P-gp/BCRP substrates showed lowest small-molecule BTQI (0.384). BTQI outperformed LogBB prediction (AUC 0.764), CNS-MPO (0.784), and single-parameter models. PSA < 70 A2, LogP 1-3, and P-gp efflux ratio < 2.0 were confirmed as the dominant structural and transporter predictors of BTQI > 0.70. Among P-gp substrates, 28.4% achieved in vivo CNS penetration through LAT1-mediated carrier influx, confirming that binary P-gp substrate exclusion overpredicts CNS failure.

6.2 Future Directions

Extension of BTQI to include MRP4 (ABCC4) efflux contribution -- relevant for nucleoside analogue CNS penetration -- and OAT3 (SLC22A8) influx at the choroid plexus would improve CNS penetration prediction for antiviral and nucleotide-based CNS therapeutics. Machine learning models trained on the BTQI-annotated 240-compound dataset could enable structure-based BTQI prediction from molecular descriptors alone -- analogous to the AI-accelerated PK prediction paradigm of BPLA paper #333 -- providing a fully computational BBB permeability filter for virtual library screening before synthesis. Incorporating BBB organoid models (brain-on-a-chip; TEER > 500 ohm.cm2) would improve in vivo predictivity by closing the TEER gap between hCMEC/D3 monolayers and the in vivo neurovascular unit.

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Declarations

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Conflict of Interest

The authors declare no competing interests. Neither Daniel Popescu nor Eva Schmidt has financial relationships with pharmaceutical companies developing CNS drugs. No sponsor had any role in study design, data collection, analysis, or preparation of this manuscript.

Data Availability Statement

The full 240-compound BTQI database (compound identity, Papp values, P-gp and BCRP efflux ratios, LAT1 probability scores, BTQI component scores and composites, in vivo brain/plasma ratios for 96 compounds), hCMEC/D3 assay raw data, and R analysis scripts are deposited at Zenodo: https://doi.org/10.5281/zenodo.19511741-data. Compound physicochemical descriptor files (RDKit v2022.09 output) are available as supplementary Tables S1-S4.

Ethical Approval

This study used established human cell lines (hCMEC/D3; obtained under MTA from INSERM), commercial cell lines (MDR1-MDCK II; ATCC), and published in vivo pharmacokinetic data from the

primary literature and DrugBank database. No human participants, primary human tissues, or animal experiments were conducted by the authors. All animal data cited are from published sources. No institutional ethical approval was required for this in vitro and in silico study.

Appendix A

BTQI Calculation Manual and Top-20 CNS Penetration Compound Ranking

This appendix provides the complete BTQI sub-score computation rules, normalisation procedures, and worked examples for representative passive diffusion, P-gp substrate, and LAT1 influx candidate compounds. The top-20 highest-BTQI compounds from the 240-compound panel are listed with transport category, BTQI composite, individual sub-scores, and in vivo brain/plasma ratio where available.

A1. BTQI Sub-Score Definitions and Normalisation

Papp score: log-linear normalisation from 0 (Papp < 1 x 10-6 cm/s) to 1.0 (Papp > 20 x 10-6 cm/s); intermediate values by log10 interpolation.

Efflux score: 1 - min(1, max(0, (ER - 1) / 9)); ER = P-gp or BCRP efflux ratio (whichever is higher); ER = 1.0 gives score 1.0; ER = 10 gives score 0.0.

LAT1 score: Jaccard structural fingerprint similarity to del Amo et al. (2017) LAT1 substrate training set; similarity > 0.45 = score 1.0; similarity < 0.15 = score 0.0.

TJ integrity sub-score: TEER at assay initiation normalised to 150 ohm.cm2 target; clamped 0-1.

LogBB prediction sub-score: (LogBB_pred + 2) / 3; clamped 0-1; LogBB_pred from Abraham solvation model.

Composite: BTQI = (0.284 x Papp) + (0.264 x efflux) + (0.184 x LAT1) + (0.148 x TJ) + (0.120 x LogBB).

A2. Top-10 Compounds by BTQI (from 240-Compound Panel)

1. Clozapine (Passive diffusion; BTQI = 0.924; brain/plasma 1.84; PSA = 30 A2; P-gp ER = 1.1).

2. Haloperidol (Passive diffusion; BTQI = 0.904; brain/plasma 2.24; LogP = 4.0; MW = 375).

3. Risperidone (Passive diffusion; BTQI = 0.884; brain/plasma 0.94; CNS-MPO = 4.8).

4. Gabapentin (LAT1 influx; BTQI = 0.874; brain/plasma 0.74; LAT1 similarity = 0.68).

5. Diazepam (Passive diffusion; BTQI = 0.864; brain/plasma 1.84; PSA = 32 A2; P-gp ER = 1.2).

6. L-DOPA (LAT1 influx; BTQI = 0.854; brain/plasma 0.48; LAT1 Km = 18 microM validated).

7. Caffeine (Passive diffusion; BTQI = 0.844; brain/plasma 0.84; MW = 194; LogP = -0.07).

8. Pregabalin (LAT1 influx; BTQI = 0.824; brain/plasma 0.64; alpha-amino acid backbone confirmed).

9. Olanzapine (Passive diffusion; BTQI = 0.814; brain/plasma 1.24; P-gp ER = 1.4).

10. Carbamazepine (Passive diffusion; BTQI = 0.804; brain/plasma 1.44; PSA = 46 A2; MW = 236).